# Purification of a recombinant functionally active *Streptococcus pneumoniae* UDP-glucose pyrophosphorylase and optimization of an assay to screen for inhibitors

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**ABSTRACT:**

The UDP-glucose pyrophosphorylase (GalU) of *Streptococcus* pneumoniae is absolutely required for the biosynthesis of capsular polysaccharide, the sine qua non virulence factor of pneumococcus. Although GalU is widely distributed, the eukaryotic enzymes are completely unrelated to their prokaryotic counterparts; therefore, we proposed that pneumococcal GalU is an important target to expand our knowledge of the mechanisms underlying capsule formation. This could contribute to the development of new therapeutic strategies to fight the pneumococcus. A recombinant form of the pneumococcal GalU was purified from *Escherichia coli* and found to be stable and catalytically active. An average of 0.6 g of active rGalU was obtained from 100 ml of culture. We describe a GalU assay that is rapid, sensitive, and easy to perform in 96-well plates. The purified enzyme was tested in the presence of several drugs with structural similarity to natural substrates of the GalU enzyme. Our results document that this colorimetric test is appropriate for screening of chemical libraries for UDP-glucose pyrophosphorylase inhibitors. This work represents a fundamental step in the search of novel antipneumococcal drugs.

**Keywords**: GalU, antipneumococcal inhibitors, high scale purification.

## 1. Introduction

The worldwide increasing resistance of bacterial pathogens to most of the currently available antibiotics fosters the research for novel therapeutic drugs. The galU gene of *Streptococcus pneumoniae* encodes a UDP-glucose pyrophosphorylase (UDPG:PP) absolutely required for capsule biosynthesis. Although this gene is highly polymorphic, there is striking sequence conservation among the UDPG:PP of both Gram-positive and Gram-negative bacteria [1]. Also, a relevant role for GalU in virulence has been recognized in other bacteria since it is required for the synthesis of UDPG, the main glucosyl donor in lipopolysaccharide and capsule biosynthesis [2–4]. Eukaryotic UDPG:PPs are completely unrelated to their bacterial counterparts, suggesting the possibility that inhibitors of the bacterial enzymes would not be harmful for the host.

Previously, the *galU* gene was cloned, and overexpressed, and GalU was biochemically characterized [5, 6] and the *galU* expression was study [7].

We describe here the expression and purification in large scale of the pneumococcal GalU enzyme. Also, we propose a method to assay GalU activity with high sensitivity and in small volumes. This method would be useful for screening of potential GalU inhibitors of the enzyme that could behave as antipneumococcal drugs.

## 2. Materials and methods

The *galU* gene was cloned into pET28a to express a His6GalU fusion protein. $His_6$GalU was purified using a NTA-Ni column.

A modification of colorimetric assay of ADPG:PP pyrophosphorylase was used [8].

## 3. Results and discussion

The recombinant GalU was overproduced in *E. coli* and purified (spec. act. 0.3 U/mg of protein). The pure enzyme was maintained stable at –20°C in 20% glycerol for at least 6 months. Several drugs with structural similarity to natural substrates of GalU were screening. We showed that after 30 min of pre-incubation with the putative inhibitor (7.5 mM), there was an activity decrease of *ca.* 50%.

## 4. Conclusions

We document the successful preparation of rGalU from *S. pneumoniae* and the optimization of a colorimetric test appropriate for the screening of UDPG:PP inhibitors. This work represents a fundamental step in the search of novel antipneumococcal drugs.

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